



# Soil life in reconstructed ecosystems: Initial soil food web responses after rebuilding a forest soil profile for a climate change experiment

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## ABSTRACT

Disrupting ecosystem components, while transferring and reconstructing them for experiments can produce myriad responses. Establishing the extent of these biological responses as the system approaches a new equilibrium allows us more reliably to emulate comparable native systems. That is, the sensitivity of analyzing ecosystem processes in a reconstructed system is improved by excluding the period when observed phenomena are primarily responses caused by establishing the experiment rather than effects of imposed treatments; achieved by determining the extent of any pulse of activity caused by preparatory procedures. A native forest soil was physically disrupted when it was collected, sieved, and then rebuilt in lysimeters in a controlled-environment study evaluating the influence of elevated atmospheric CO<sub>2</sub> concentration and elevated atmospheric temperature on the reconstructed soil that was planted with Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings. Generally, soil food web populations responded in two phases during the exposure as indicated by preliminary evaluation of the 4.5-year dataset. Also, previous work indicated that relatively elevated soil CO<sub>2</sub> effluxes occurred during the first phase, suggesting that food web populations may have responded to carbon sources made available when the soil was harvested and its profile reconstructed in the lysimeters. Results are presented for bacterial and fungal biomass, numbers of protozoa and nematodes to gain insight on whether the first phase responses are attributable to the acute stress of physically disrupting the soil. We found clear relationships between changes in predator and prey populations. A prominent spike for many of the food web populations occurred the year after the climate exposures began. Except for total bacterial biomass and total fungal-hyphae biomass, overall food web responses generally were unrelated to treatments. It appears that initial food web population responses were related to increased availability of soil carbon caused by establishing the experiment. Our results provide insights into determining the length of time to maintain reconstructed forest ecosystems before responses are observed related to experimental treatments. It appears that as long as 3 years elapsed before the soil food web appeared to recover from the acute physical disturbance; 1 year of recovery prior to commencing the climate exposures to allow the soil to rest after it was reconstructed, plus approximately the first 2 years of maintaining the climate treatments. Accounting for consequences of such periods of adjustment is critical for forecasting whether comparable natural ecosystems will be net sources or sinks of elevated concentrations of atmospheric CO<sub>2</sub>.

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## 1. Introduction

Disrupting ecosystems can produce a variety of transient and longer term responses. These may include system energetics,

nutrient cycling, the structure of a community, and a number of general system-level characteristics, e.g., the reversal of autogenic successional trends, increased tendencies for parasitism and other negative interactions, and decreased tendencies for mutualism and other positive interactions (Odum, 1985). For studies examining the fate of C in response to climate change drivers, the amount of soil organic matter (SOM) in a physically disturbed soil initially can rapidly decrease (Davidson and Ackerman, 1993), and nitrogen (N) mineralization and nitrification can be stimulated (Johnson et al., 1995 and references therein).

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Whether the reconstructed system emulates the comparable native system is one issue to address in all studies involving reconstructed ecosystems. Johnson et al. (1995) examined this question while studying effects of CO<sub>2</sub> and N on growth of Ponderosa pine (*Pinus ponderosa* Laws.) seedlings. They found that their results for the controlled-environment chamber study, which utilized 0.027 m<sup>3</sup> pots, contradicted those obtained in the comparable field study. Increased rates of N mineralization became evident soon after the experiment was initiated resulting in a redesign of the sampling protocol to address whether the pot systems would recover during the 32-week experiment.

Odum (1985) suggested that disturbance will lead to enriched food webs as resources become available or the environment becomes more favorable for the enriched food web. This does not imply that an enriched food web is preferable, but rather that more microbial food resources become available. These food sources may be derived from plant material killed by the disturbance or from organic matter previously unavailable to microbes. As Odum (1985) postulated, such a condition can be indicated by increased community respiration as the system “pumps out” the short-term disorder (Westerman and Kurtz, 1973; Hoosbeek et al., 2004; Hirotsugu et al., 2007; Ohm et al., 2007), i.e., the system comes to equilibrium again. Lin et al. (2001), working in our chambers, found evidence generally consistent with this response. Using stable isotope signatures of soil CO<sub>2</sub> efflux and of its component sources they proportioned total efflux into its sources. The greatest relative changes in proportional contributions occurred in the second year, specifically from oxidation of native SOM that was present when the native soil was initially collected. The authors speculated that increased total CO<sub>2</sub> efflux resulted from enriched food web conditions caused by physically disrupting soil aggregates as the soil was prepared and placed into the lysimeters. They cautioned that such responses, unless understood and noted, can alter inferences drawn from experiments if they are not allowed to recover from disturbances caused by initiating the experiment.

Monitoring food web organisms can reflect responses to various environmental drivers, from daily or weekly (e.g., activity of bacteria and fungi for fluctuations in temperature, moisture, nutrient availability) to seasonally (total bacterial and fungal biomass for carbon availability, temperature and moisture) (Ingham and Thies, 1996). One of the overarching goals of the 4.5-year climate exposure project, of which the research reported herein is a component, was to determine whether the reconstructed soil-seedling systems would be net sources or sinks of C. Examining soil food web dynamics would contribute to meeting this goal by understanding temporal patterns of the detrital food web to create and stabilize SOM. Odum (1985) further suggested that systems may respond differently to chronic stress that continues for an extended period compared with responses to acute stress that are followed by a period of recovery. Our experimental system experienced both an acute stress, i.e., the harvesting of the soil and its placement in the lysimeters, and a chronic stress, i.e., the climate treatments. It would be helpful to any project where the soil system has been perturbed, to be able to assess responses related to such an acute stress as well as assess responses related to the chronic stress. Preliminary observations of the entire food web dataset from the 4.5-year climate exposure period yielded two distinct phases for responses of the soil food web populations; the first phase lasting approximately 2 years (data not shown). Responses of soil food web populations during this initial 2-year period of the climate exposure regimen, plus the procedures used in the year prior to commencing the climate treatments to establish the experimental ecosystems, are the focus of the work reported herein. Results are presented for bacterial and fungal biomass, numbers of protozoa and nematodes. This

information will aid in identifying the magnitude of, and length of time for, early system responses that may reflect effects of the acute stress of disturbing the soil. This information also will contribute to estimating the length of time needed to maintain experiments using reconstructed ecosystems to understand the variety of responses observed.

## 2. Materials and methods

### 2.1. Experimental facility, soil, seedlings and litter

Twelve SPAR (Soil Plant Atmosphere Research) chambers were used to contain the soil and vegetation, and to provide the altered climate conditions [see Tingey et al. (1996) for details about chamber performance]. Briefly, each chamber has a 1 m × 2 m footprint (2 m<sup>2</sup>) and collectively, the 12 independently monitored and controlled chambers form a portion of an environment-tracking, sun-lit, controlled-environment facility that links atmospheric, plant and soil processes. Each chamber consists of a 1 m deep lysimeter (2 m<sup>3</sup> vol.), and an aboveground aluminum-framed canopy (1.5 m tall at the back, sloping to 1.2 m tall at the front, 3.18 m<sup>3</sup> vol.) that is covered with a 3 mil clear Teflon<sup>®</sup> film. An important characteristic of the facility is that it can, in real time, simulate natural seasonal and diurnal changes in atmospheric CO<sub>2</sub>, air and soil temperatures, vapor pressure deficit (VPD), and soil moisture.

The soil was derived from a glacially worked, volcanic parent material and is classified as a coarse-loamy, mixed, frigid, Typic Hapludand. This soil represents about 30% of the soils supporting growth of Douglas-fir in the Oregon Cascade Mountains (personal communication, Douglas Shank, USDA Forest Service, Willamette National Forest). The soil has three master mineral horizons [A horizon (~10 cm thick), a B horizon (~60 cm thick), and a C horizon (~20 cm thick)] and a 6 cm thick forest floor litter surface layer (O horizon). Since the B horizon is large, it was treated as two horizons (B1 and B2, 30 cm each) as it was placed into the lysimeters and instrumentation was installed.

The soil was excavated by horizon adjacent to an old-growth Douglas-fir stand situated at 1220 m in the Cascade Mountains directly east of Corvallis (see Johnson et al., 2006 for more details). Soil collection and placement in the lysimeters was accomplished in two phases. This was done so that the majority of the soil could rest for a period before planting the seedlings, and recover from disturbance caused by its excavation, sieving (>2.5 cm material removed) and placement in the chambers. In phase one, within a few days of arriving in Corvallis in summer 1992, the sieved soil horizons and associated instrumentation and samplers were installed by layers and then each layer tamped to adjust the soil to the native bulk density. The lysimeters were not covered with the chamber canopies until the seedlings were planted (1993). In the interim the soil received only ambient precipitation (i.e., summer drought conditions with increased precipitation occurring from fall through spring).

In phase one, cover crops were planted in the reconstructed partial soil profile to aid in regaining macrostructure, and to reduce the amount of any mineralized nitrogen that may have been produced by relocating the soil. Buckwheat (*Fagopyrum esculentum* Moench) was planted shortly after reconstructing the soil profile and was allowed to grow until shoots were harvested and removed in late Fall 1992. Next, a mixture of faba bean (*Vicia faba* L.) and hairy vetch (*Vicia villosa* Roth) was planted and was allowed to grow until Spring 1993 when shoots were harvested.

Phase two of reconstructing the soil profile occurred in summer 1993, and began 48 h prior to planting the Douglas-fir seedlings when fresh A horizon was collected adjacent to the soil collection site used previously. The A horizon provided fresh native soil flora

and fauna populations. Prior to installing the A horizon, the top 2 cm of the B1 horizon in the lysimeters were excavated to remove any debris that may have accumulated, and reduce aboveground plant detritus and a portion of any residual root originating from the cover crops. Thus, at the time the climate change exposures commenced in summer 1993, the B1, B2 and C horizons had rested in place for 1 year and had supported three cover crops, while the A horizon was recently installed.

Commercially produced, open-pollinated, 1 + 1 (1 year in the seed bed followed by 1 year in the nursery bed), Douglas-fir seedlings were used. The seedlings were germinated from an aggregated, blended seed stock that originated from 5 low-elevation (300–460 m) seed zones in the Oregon Cascade and Coastal Mountain Ranges that are near and surround Corvallis (see Rygielwicz et al., 2000 for more details). Fourteen seedlings were planted in each chamber with roots extending through the A horizon and into a few cm of the B1 horizon as described by Olszyk et al. (1998). Seedlings were planted in three rows of 5, 4 and 5 seedlings, respectively, creating triangular locations formed by three seedlings, from which soil cores would be obtained during the exposure period (see below for more details about harvesting the soil cores).

Within 1 week after planting the seedlings, the O horizon was collected from a Douglas-fir stand adjacent to the soil collection site, sieved (2.5 cm) and then samples were taken to determine moisture content to calculate dry mass. Within two days of collecting the O horizon, a forest floor was recreated in each lysimeter by adding a calculated dry mass of sieved O horizon, equivalent to 6 cm thickness that was found at the soil collection site.

## 2.2. Experimental design and treatments

The controlled-climate experiment consisted of a  $2 \times 2$  factorial design, with 3 replicates (chambers). Each climate exposure regimen was based on measured ambient conditions assessed at a meteorological station located adjacent to the chamber facility. The treatments were (1) ACAT: ambient–ambient (i.e., ambient atmospheric  $\text{CO}_2$  concentration and ambient atmospheric temperature, based on the assessment done at the meteorological station); (2) ECAT: elevated  $\text{CO}_2$  (i.e., target of  $0.2 \text{ kg m}^{-3}$  above ambient) and ambient temperature; (3) ACET: ambient  $\text{CO}_2$  and elevated temperature (i.e., target of  $4^\circ\text{C}$  above ambient); and (4) ECET: elevated–elevated (i.e., elevated  $\text{CO}_2$  and elevated temperature). Implementing the climate treatments began in late summer 1993, shortly after the chamber canopies were placed on the lysimeters. The performance of the facility was monitored constantly and provided climate exposures within nominal conditions as described by Tingey et al. (1996). During the first 2 years of the exposure (1993–1994) the ambient atmospheric  $\text{CO}_2$  concentration at the facility assessed at the meteorological station averaged  $0.35 \text{ kg m}^{-3}$ . Overall, the facility provided  $0.18 \text{ kg CO}_2 \text{ m}^{-3}$  and  $3.8^\circ\text{C}$  above the ambient set-points for the elevated climatic conditions. In order to achieve the elevated temperature treatment (i.e., maintain constant VPD between the temperature treatments), the dew point in all chambers was maintained such that the dew point depression was similar to the values measured at the meteorological station.

## 2.3. Care of seedlings, soil and litter

The climate exposures began with the nutrients, soil flora and mesofauna present in the sieved soil or associated with the seedlings as received from the nursery. Soil nitrogen concentrations are low: total N is  $<0.01 \text{ g kg}^{-1}$ , and concentrations of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in soil solutions are below detection limits (0.04 and

$0.10 \text{ g m}^{-3}$ , respectively). As atmospheric nutrient inputs in high-elevation Cascade Mountain forests in Oregon are minimal ( $0.56\text{--}1.38 \text{ kg N ha}^{-1} \text{ y}^{-1}$ , National Atmospheric Deposition Program, 1998), no mineral fertilizer was added during the study. Some amount of exogenous N was added through N-fixation by free-living bacteria (mean maximal rate measured during the experiment was approximately  $1 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) and by N-fixing symbionts colonizing the cover crops (data not shown). Also, to aid in maintaining endogenous populations of litter and soil insects and microorganisms, no synthetic-chemical pesticides were used. On an as-needed basis, combinations of predatory insects, insect parasites and a soap solution applied directly onto herbivores were used to control outbreaks of aboveground herbivory.

A representative Pacific Northwest xeric soil moisture regimen (i.e., soils at field capacity in winter and drier during the summer, described below) was followed, except for summer 1993. The first summer dry period was not imposed so the newly planted seedlings could more readily survive the first season. Instead seedlings were watered weekly, with the same amount of water added to each chamber, using a hand-held watering wand. Sufficient reverse osmosis (RO) water was added to keep moisture just below field capacity in the portion of the soil where roots were found (using minirhizotron tubes), with the amount of water added being determined using time-domain reflectometry (TDR) probes deployed in each soil horizon (Johnson et al., 2006).

Starting Fall 1993 the xeric soil moisture regimen was imposed. Target soil moisture was based on seasonal soil moisture determined for the local area by Griffiths et al. (1990). We developed our seasonal watering regimen using this general pattern and long-term data (30 y) of monthly precipitation and potential evapotranspiration (PET) for the Corvallis area. Four seasonal soil moisture conditions were maintained: (1) wet winter, i.e., soil water was maintained at or near field capacity; (2) spring–summer dry-down i.e., soil water was slowly reduced as the rate of water removed from the soil increased from rising PET rates and declining amounts of water added; (3) dry summer, the period of lowest soil moisture ( $\sim 50\%$  of field capacity), was maintained for approximately two months; and (4) rapid fall rewetting which corresponded to the start of the wet season where water additions increased in conjunction with declining PET rates. The amount of water added to all chambers was based on the amount of water required to keep the soil under the ACAT treatment at or near the target soil moisture condition. The RO water was provided at the top of the seedling canopy using a hand-held wand.

## 2.4. Soil collection and enumeration of soil food web populations

Beginning Fall 1993, and continuing semi-annually (spring and fall), one full-soil profile, soil core (5-cm i.d.) per chamber was taken from the center of a triangle defined by three seedlings located on the non-instrumented half of each lysimeter. Each triangle was sampled once during the exposure period and the order of sampling the triangles in the chambers during the experiment was randomly assigned. The same corresponding triangle, within each chamber, was sampled in all chambers. Dates to collect the soil cores each spring and fall were selected based on edaphic conditions and seedling phenological events. Sampling in the spring generally occurred sometime in March under conditions of high soil moisture and prior to bud burst. Fall sampling generally occurred sometime in September prior to the start of the rewetting phase based on local climatic conditions.

The soil from the cores from each of the mineral horizons was handled separately after the O horizon was removed. The soil was sieved (2 mm openings) to separate soil from roots and coarse fragments. The freshly sieved soil was divided into several aliquots

for physical, chemical and various biological and biochemical analyses. Active bacterial and fungal-hyphae biomass measured using fluorescein diacetate (FDA) stain, and length of active and total fungal hyphae were determined following the procedures of Ingham and Klein (1984), Ingham and Horton (1987) and Lodge and Ingham (1991), and the biovolume-to-biomass conversion rates of Van Veen and Paul (1979) were applied as necessary. Total bacterial biomass was determined using direct count microscopy and fluorescein isothiocyanate (FITC) stain, based on the procedures of Babuik and Paul (1970), and then applying the conversion rates of Van Veen and Paul (1979). Protozoan populations (flagellates, amoebae, and ciliates) were calculated using most probable number (MPN) tables to convert presence data obtained by differential interference contrast (DIC) microscopy on serial-diluted soil samples (Ingham, 1993; Darbyshire et al., 1974; Dindal, 1990). After the protozoa in the diluted samples were allowed to incubate on a substrate of soil extract agar, the samples were scored for the presence of amoebae, flagellates and ciliates according to the descriptions of Lee et al. (1985). MPN values were converted to mass equivalents according to the conversion values of Darbyshire et al. (1974). Nematodes first were extracted from the soil using Baerman extractors according to the procedures of Anderson and Coleman (1977). Enumeration was done within 2–4 weeks after extraction using refrigerated, non-fixed specimens and a dissecting microscope. If the time until enumeration would be longer than 4 weeks, the extracted nematodes were heated killed (65 °C) and then preserved in TAF (triethanolamine, distilled water, 35% formalin). A subsample of enumerated nematodes was further observed using DIC microscopy and identified to at least genus using criteria outlined in the keys of Bongers (1988), Goody (1963), and Mai and Lyon (1975).

Data, numbers or biomass per g dry mass of soil obtained from the enumeration procedures, were converted to per m<sup>3</sup> using the calculated total dry mass of soil of the subject horizon placed into the 2 m<sup>2</sup> lysimeters and the thickness of the horizon. Chamber values denote presence per m<sup>3</sup> in the soil profile obtained using the proportionally weighted results of the four horizons and the 88 cm total thickness of the soil profile.

### 2.5. Statistical analyses

The experiment followed a factorial treatment design with four factors: atmospheric CO<sub>2</sub> concentration treatment, atmospheric temperature treatment, time and soil horizon. The experimental design is a multilevel, hierarchical design. It was analyzed using a linear mixed model, accounting for the multilevel structure with multiple error terms (Littell et al., 2006). At the first level, “between-chamber”, the CO<sub>2</sub> and temperature treatments were applied to each of the 12 chambers, following a complete balanced design with 3 replicates per treatment combination. At the second level, “within-chamber”, soil cores were sampled at different dates to assess the time evolution of the response variables. Bacterial, fungal and protozoan populations were analyzed using data obtained from four sampling dates. Data for nematode populations were not included in the statistical analyses for the third sampling date (Fall 1994) as these data were not available. At the third level, “within soil core”, population levels were assessed in each of the four mineral horizons. The final linear model included three random effects, one for each of the three levels. For the full-chamber analyses, data from the four horizons were combined as described above to obtain one value per chamber and time, therefore reducing the number of levels to two. Residual plots showed that the assumptions of normality, linearity and constant variance were not met, indicating a highly skewed distribution and multiplicative error structure. A logarithmic transformation corrected the problems for all variables except total fungal biomass, for which a square root transformation was

sufficient to meet the assumptions. This was achieved by taking the natural log of the values after adding one half of the lowest, non-zero value for the variable, thus allowing zero values to be included in the analysis. The median is considered a better summary of central tendency than is the mean when the distribution of the data is skewed. Therefore, our reported summaries of the transformed data are reported as estimated medians and treatment effects as ratios of population medians (Ramsey and Schafer, 1997, p. 67). When a factor was statistically significant ( $p \leq 0.05$ ), multiple comparisons among treatment means followed using  $p \leq 0.05$  to indicate significant differences. The family-wise error rate was controlled with the SIMULATE option in SAS PROC MIXED. All analyses were carried out with SAS software version 9.1 (SAS Institute, Cary, NC, USA).

## 3. Results

### 3.1. Bacterial biomass

#### 3.1.1. Total bacterial biomass

CO<sub>2</sub> treatment, time and soil horizon affected bacterial biomass (Table 1). There was no evidence that the atmospheric temperature treatment affected biomass. As there were no statistically significant interactions found, biomass varied uniformly among climate treatments, through time and among soil horizons. Overall, elevated atmospheric CO<sub>2</sub> (EC) increased the median total biomass by approximately 22% (95% CI from 1 to 48%) compared with the median biomass estimated for the ambient CO<sub>2</sub> (AC) treatment; 3.57 vs. 2.92 g m<sup>-3</sup>, respectively, all soil horizons (i.e., chamber value) and sampling times pooled. Total biomass (chamber value) was comparatively low (1.77 g m<sup>-3</sup>) in Fall 1993 (F93). Pairwise comparisons indicated that total biomass was greatest (7.07 g m<sup>-3</sup>) at the second measurement period, Spring 1994 (Sp94). It was significantly different from the biomass in F93, Fall 1994 (F94) and Spring 1995 (Sp95), which were not different among themselves. Median total biomass in Sp94 is estimated to be 4.0, 2.3 (3.15 g m<sup>-3</sup>) and 2.6 (2.75 g m<sup>-3</sup>) times greater than at F93, F94 and Sp95, respectively (chamber values, Fig. 1). Median total bacterial biomass was greatest in the A horizon (4.7 g m<sup>-3</sup>), followed by values for the remaining three horizons which were not significantly different from each other (2.8, 2.7 and 3.1 g m<sup>-3</sup> for the B1, B2 and C, respectively). Overall, biomass in the A horizon ranged from 1.5 to 1.7 times greater than amounts calculated for the three lower horizons.

#### 3.1.2. Active bacterial biomass

Unlike the responses for total bacterial biomass, there was no evidence that active bacterial biomass differed between CO<sub>2</sub> treatments (Table 1). Overall, the patterns of active biomass in the lysimeters tracked those of total biomass. Similar to differences among total biomass, active mass significantly varied by time and among soil horizons, and there was not a significant effect of the temperature treatments. Also, as there were no significant interactions found, active biomass varied uniformly through time and among soil horizons. Pairwise comparisons indicated that estimated median active biomass was greatest in Sp94 (2.4 g m<sup>-3</sup>), which was 2.8 (0.86 g m<sup>-3</sup>), 3.3 (0.71 g m<sup>-3</sup>) and 3.0 (0.78 g m<sup>-3</sup>) times greater than the biomass estimated for F93, F94 and Sp95, respectively (Fig. 1, chamber values). Biomass was similar among the latter three sampling times. Estimated median active biomass was greatest in the A (1.9 g m<sup>-3</sup>) and significantly different from values for the remaining three horizons (1.0, 0.77 and 0.73 g m<sup>-3</sup> for the B1, B2, and C). Active biomass in the A was 1.8, 2.4 and 2.5 times values calculated for the other three horizons, respectively as before. Biomass in the B1 differed from values in the B2 and C; the latter horizons supporting similar amounts of active bacterial biomass.



**Table 1**

Results of analyses of variance (ANOVA) on log transformed data of total and active bacterial biomass obtained from soil samples harvested twice annually between Fall 1993 and Spring 1995 in an elevated atmospheric CO<sub>2</sub> and atmospheric temperature experiment.

Factor <sup>a</sup>	Num DF	Den DF	p-Values			
			Bacterial biomass			
			Total		Active	
			By horizon	Chamber	By horizon	Chamber
CO <sub>2</sub>	1	8	<b>0.033</b>	<b>0.039</b>	0.529	0.641
Temp	1	8	0.864	0.954	0.637	0.654
CO <sub>2</sub> × Temp	1	8	0.697	0.884	0.863	0.846
Time	3	24	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
CO <sub>2</sub> × Time	3	24	0.753	0.644	0.942	0.958
Temp × Time	3	24	0.843	0.838	0.639	0.641
CO <sub>2</sub> × Temp × Time	3	24	0.922	0.927	0.838	0.835
Hor	3	96	<b>&lt;0.0001</b>	–	<b>&lt;0.0001</b>	–
CO <sub>2</sub> × Hor	3	96	0.419	–	0.199	–
Temp × Hor	3	96	0.606	–	0.619	–
CO <sub>2</sub> × Temp × Hor	3	96	0.448	–	0.339	–
Time × Hor	9	96	0.142	–	0.241	–
CO <sub>2</sub> × Time × Hor	9	96	0.106	–	0.543	–
Temp × Time × Hor	9	96	0.672	–	0.570	–
CO <sub>2</sub> × Temp × Time × Hor	9	96	0.899	–	0.203	–

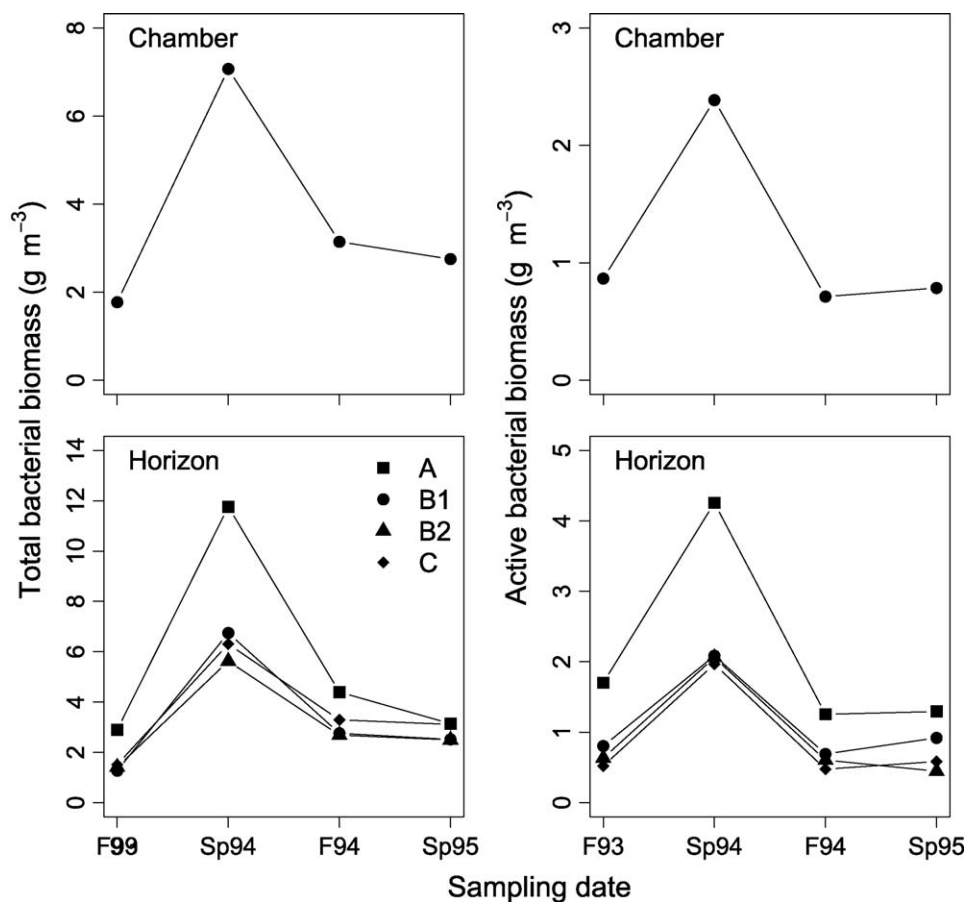
<sup>a</sup> Temp, temperature; Hor, soil horizon; Num DF, degrees of freedom of the numerator; Den DF, degrees of freedom of the denominator; By horizon indicates ANOVA results for the four soil horizons analyzed separately, Chamber denotes ANOVA results for data of the four soil horizons combined to represent the soil profile reconstructed in the lysimeters, boldface type indicates significance at  $p \leq 0.05$ .

### 3.2. Protozoa

#### 3.2.1. Amoebae

CO<sub>2</sub>, time and horizon affected the numbers of amoebae during the exposure period (Table 2). While the effect of temperature was

not statistically significant, the CO<sub>2</sub> × temperature interaction was significant in the horizon analysis, almost significant in the chamber analysis and there was a significant CO<sub>2</sub> × time interaction indicating a differential CO<sub>2</sub> effect on amoebae as the exposure continued and depending on the temperature treatment.



**Fig. 1.** Estimated median (12 observations/point) total (left) and active (right) bacterial biomass. Data are for the soil profile (chamber) and for each master horizon (A, B1, B2 and C). Notes: (1) Even though CO<sub>2</sub> treatment significantly affected total bacterial biomass, results are presented by horizon as there were no significant interactions found between CO<sub>2</sub> and any other factor (Table 1). (2) Ranges of density values on the y-axes may differ among the horizons. They also can vary between chamber and horizon graphs as density values for the chamber are not the addition of each horizon value.

**Table 2**

Results of analyses of variance (ANOVA) on log transformed data of number of amoeba and flagellates. All other notations as described in Table 1.

Factor	Num DF	Den DF	p-Values			
			Amoebae		Flagellates	
			By horizon	Chamber	By horizon	Chamber
CO <sub>2</sub>	1	8	<0.0001	<0.0001	<0.0001	<0.0001
Temp	1	8	0.178	0.166	0.951	0.969
CO <sub>2</sub> × Temp	1	8	0.025	0.060	0.110	0.046
Time	3	24	<0.0001	<0.0001	<0.0001	<0.0001
CO <sub>2</sub> × Time	3	24	<0.0001	<0.0001	<0.0001	<0.0001
Temp × Time	3	24	0.822	0.767	0.682	0.340
CO <sub>2</sub> × Temp × Time	3	24	0.828	0.807	0.513	0.538
Hor	3	96	<0.0001	–	<0.0001	–
CO <sub>2</sub> × Hor	3	96	0.753	–	0.288	–
Temp × Hor	3	96	0.900	–	0.976	–
CO <sub>2</sub> × Temp × Hor	3	96	0.263	–	0.805	–
Time × Hor	9	96	0.084	–	0.013	–
CO <sub>2</sub> × Time × Hor	9	96	0.652	–	0.403	–
Temp × Time × Hor	9	96	0.139	–	0.702	–
CO <sub>2</sub> × Temp × Time × Hor	9	96	0.129	–	0.040	–

In general, the patterns of responses for amoeba mimicked the general responses found for total and active bacterial biomass, i.e., maximum counts were found in Sp94 relative to calculated median values for samples obtained prior to or after that date. Overall, amoebae counts under AC were lower than under the EC treatments ( $58 \times 10^6$  and  $170 \times 10^6 \text{ m}^{-3}$ , respectively). The CO<sub>2</sub> × temperature interaction is described as: for the AC treatments, amoebae decreased with increased temperature ( $80 \times 10^6 \text{ m}^{-3}$  under AT treatments vs.  $42 \times 10^6 \text{ m}^{-3}$  under ET treatments), while under the EC treatments amoebae counts increased with increased temperature ( $161 \times 10^6 \text{ m}^{-3}$  under AT treatments vs.  $180 \times 10^6 \text{ m}^{-3}$  under ET treatments). The CO<sub>2</sub> × time interaction appeared related to how CO<sub>2</sub> affected amoeba numbers at the beginning and end of the exposure (F93 and Sp95) vs. the effect of CO<sub>2</sub> on amoebae at Sp94 and F94 (Fig. 2, chamber values). Amoebae were not detected in F93 in the AC treatments whereas at this time the median number of amoebae in the EC treatments was  $178 \times 10^6 \text{ m}^{-3}$ . A similar relative pattern was found in Sp95 with comparable treatment values of  $31 \times 10^6$  and  $136 \times 10^6 \text{ m}^{-3}$ . Amoeba numbers at the two intermediate sampling times reveal both the CO<sub>2</sub> × time and the CO<sub>2</sub> × temperature interactions (Fig. 2, chamber values). Amoeba numbers in Sp94 were greatest and similar in the ACAT and ECET treatments ( $454 \times 10^6$  and  $472 \times 10^6 \text{ m}^{-3}$ , respectively), followed by lower numbers in the ECAT and ACET ( $313 \times 10^6$  and  $271 \times 10^6 \text{ m}^{-3}$ , respectively). Amoebae declined in numbers, respectively by treatment, from Sp94 to F94. However, the ECET had the greatest relative decline in number of amoebae leaving only the ACAT significantly greater than the other treatments in F94. Overall, the A horizon had the greatest estimated median number of amoeba ( $122 \times 10^6 \text{ m}^{-3}$ ), followed by values for the remaining three horizons that were not significantly different from each other (68, 61 and  $49 \times 10^6 \text{ m}^{-3}$  for the B1, B2 and C, respectively). Counts in the A horizon ranged from 1.8 to 2.5 times greater than values calculated for the three lower horizons.

While it appears that the numbers of amoebae in some climate treatments may have peaked at different times in the various horizons, none of the interactions involving horizon were statistically significant (Table 2).

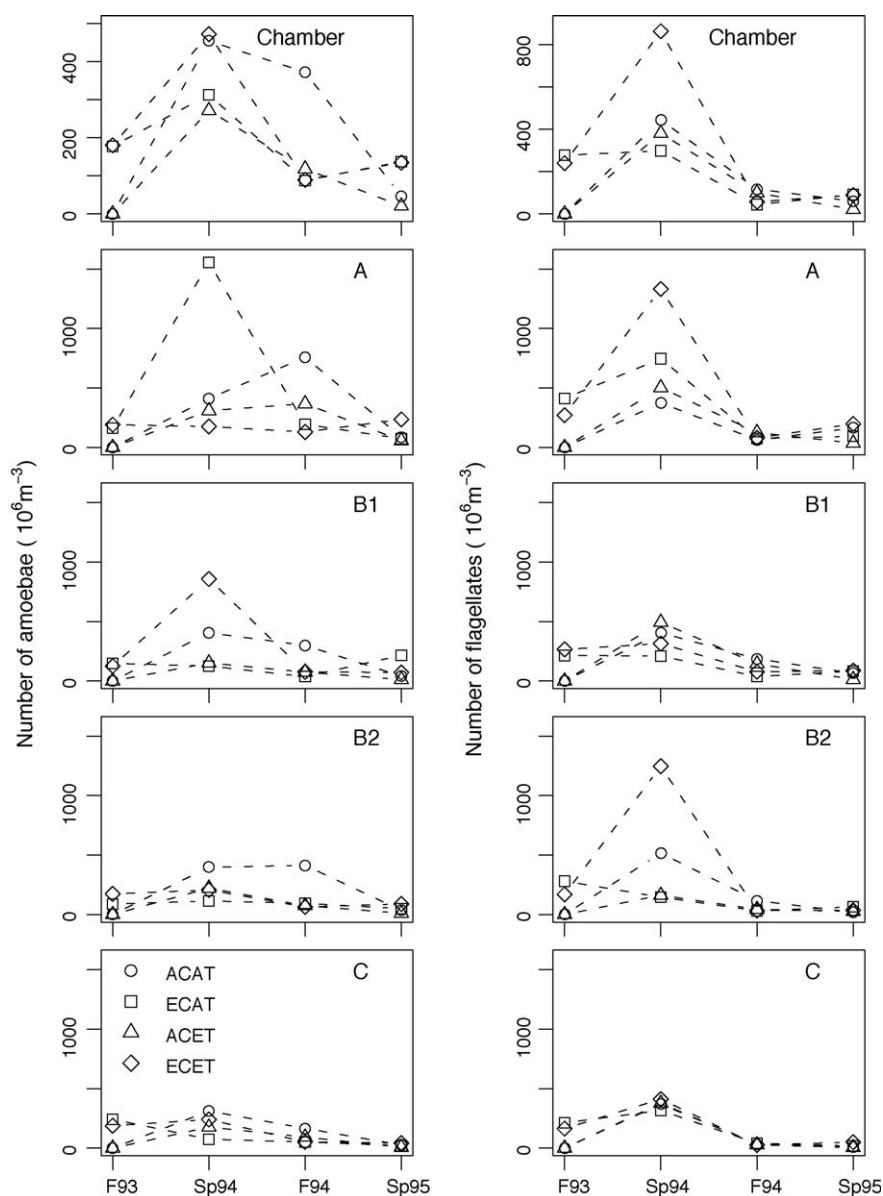
### 3.2.2. Flagellates

CO<sub>2</sub>, time and horizon affected the numbers of flagellates (Table 2). There was a significant CO<sub>2</sub> × time interaction indicating a differential CO<sub>2</sub> effect on flagellate numbers as the exposure continued. Also, time differentially affected flagellate numbers depending on the horizon (time × horizon interaction). The four-

way interaction of CO<sub>2</sub> × temperature × time × horizon was also significant suggesting that flagellate numbers in the various horizons and during the exposure were differentially affected by the two climate treatments. Overall, flagellate counts in F93 were low relative to peak values found in Sp94, after which counts returned to levels similar to those found at the beginning of the exposure (Fig. 2). The interaction between the CO<sub>2</sub> treatments and time was similar to the pattern found for amoebae, i.e., that the difference was most notable between F93 and Sp94. Flagellates were not found in F93 under the AC treatments, while the estimated median number for the EC treatments was  $258 \times 10^6 \text{ m}^{-3}$  (Fig. 2, chamber values). Between F93 and Sp94 flagellate numbers in the AC treatments increased relatively more steeply (from 0 to  $411 \times 10^6 \text{ m}^{-3}$ ) compared with numbers under the EC treatments ( $258 \times 10^6$  to  $508 \times 10^6 \text{ m}^{-3}$ ). The highly significant CO<sub>2</sub> × time interaction appears to be caused by the very different values between the elevated CO<sub>2</sub> treatments in Sp94 ( $863 \times 10^6$  vs.  $298 \times 10^6 \text{ m}^{-3}$  for the ECET and ECAT treatments, respectively). Patterns of changes in flagellate numbers during the exposure were not similar across horizons, with Sp94 being the most notable sampling time where the relative ranking of the EC and AC treatments was different in each horizon. It appears that the significant four-way interaction relates primarily to changes in relative numbers in Sp94 (Fig. 2, chamber values) and was largely due to responses in the B2 horizon (Fig. 2). The temperature effect from F93 to F94 in the B2 was unlike the temperature effect found in the other horizons; in the A, B1 and C horizons, flagellates responded relatively similarly under each temperature treatment within each CO<sub>2</sub> treatment. However, in the B2, the temperature effect was not uniform across CO<sub>2</sub> treatments. Flagellate numbers increased more between F93 and Sp94 under both of the matched-level climate treatments, i.e., the ECET and ACAT treatments compared with the two mixed-level (ACET and ECAT) climate treatments such that the estimated median number of flagellates was greater under the matched-level treatments ( $1247 \times 10^6$  and  $516 \times 10^6 \text{ m}^{-3}$ ; ECET and ACAT, respectively) compared with estimated numbers of flagellates for the mixed-level treatments ( $162 \times 10^6$  and  $146 \times 10^6 \text{ m}^{-3}$ ; ACET and ECAT, respectively).

### 3.2.3. Ciliates

Ciliates were not found for most sampling times, under most treatments. Among the sampling times when they were found, the overall response was idiosyncratic with respect to treatments. The largest individual counts of ciliates during the exposure ranged



**Fig. 2.** Estimated median (3 observations/point) counts of amoeba (left) and flagellates (right). Data are for the four climate treatments for the soil profile (chamber) and for each of the master horizons since significant differences were found among climate treatments and horizons. *Notes:* Ranges of y-axis values are different among the plots.

between 4 and  $5 \times 10^6 \text{ m}^{-3}$  (chamber value). Otherwise when present, chamber values for ciliates were less than  $10^6 \text{ m}^{-3}$ .

### 3.3. Fungal-hyphae biomass

#### 3.3.1. Total fungal-hyphae biomass

The significant four-way interaction of  $\text{CO}_2 \times \text{temperature} \times \text{time} \times \text{horizon}$  for total fungal-hyphae biomass makes interpreting responses complicated (Table 3). The interaction indicates that at least one treatment combination is different from the other 63 treatments ( $2 \text{ CO}_2 \text{ levels} \times 2 \text{ temperature levels} \times 4 \text{ sampling times} \times 4 \text{ horizons} = 64 \text{ treatments}$ ). Given that many significant interactions were found, we present results of horizon-by-horizon analyses of responses to elucidate any patterns (Table 3). Note that horizon was highly significant which indicates that the estimated median amounts of total fungal-hyphae biomass differed among horizons (Fig. 3). Biomass in the A horizon was greatest ( $524 \text{ g m}^{-3}$ ) followed by lower values in the three remaining horizons which did not differ among themselves (71, 43 and

$34 \text{ g m}^{-3}$ , B1, B2 and C, respectively, treatment and sampling time data pooled for all values). However, the very significant time  $\times$  horizon and four-way interactions suggest that each horizon was affected differently by the treatments during the course of the exposure. Overall, only time affected total fungal-hyphae biomass in the B2 and C horizons (Table 3). For the A and B1 horizons the three-way  $\text{CO}_2 \times \text{temperature} \times \text{time}$  interaction was significant suggesting that each climate treatment affected total fungal-hyphae biomass differently as the exposure progressed.

**3.3.1.1. A horizon.** Neither climate treatment main effect was found significant. The interaction between the climate treatments was nearly significant (Table 3). The significant  $\text{CO}_2 \times \text{temperature} \times \text{time}$  interaction indicates that  $\text{CO}_2$  and temperature effects were not uniform during the exposure. Namely, a seasonal oscillation in hyphal biomass may have occurred under each of the four climate treatments where the oscillation appears to be less chaotic with respect to the climate treatments early during the exposure period. Even so, the relative pattern of oscillation with respect to any

**Table 3**

Results of analyses of variance (ANOVA) on (A) square root transformed data of total fungal-hyphae biomass and log transformed data of active fungal-hyphae biomass, and (B) ANOVA results for soil horizons on data of total fungal-hyphae biomass. All other notations as described in Table 1.

Factor	Num DF	Den DF	p-Values			
			Fungal-hyphae biomass			
			Total		Active	
			By horizon	Chamber	By horizon	Chamber
(A)						
CO <sub>2</sub>	1	8	0.777	0.696	0.725	0.994
Temp	1	8	0.070	0.106	0.156	0.204
CO <sub>2</sub> × Temp	1	8	0.094	0.065	0.770	0.696
Time	3	24	<b>&lt;0.0001</b>	<b>0.039</b>	<b>0.001</b>	<b>&lt;0.0001</b>
CO <sub>2</sub> × Time	3	24	0.863	0.970	0.933	0.635
Temp × Time	3	24	0.265	0.255	0.431	0.172
CO <sub>2</sub> × Temp × Time	3	24	<b>0.019</b>	<b>0.030</b>	0.495	0.091
Hor	3	96	<b>&lt;0.0001</b>	–	<b>&lt;0.0001</b>	–
CO <sub>2</sub> × Hor	3	96	0.278	–	0.230	–
Temp × Hor	3	96	0.081	–	0.658	–
CO <sub>2</sub> × Temp × Hor	3	96	<b>0.001</b>	–	0.465	–
Time × Hor	9	96	<b>&lt;0.0001</b>	–	<b>0.036</b>	–
CO <sub>2</sub> × Time × Hor	9	96	0.942	–	0.110	–
Temp × Time × Hor	9	96	0.577	–	0.547	–
CO <sub>2</sub> × Temp × Time × Hor	9	96	<b>0.001</b>	–	0.095	–
Total fungal-hyphae biomass						
Horizon						
			A	B1	B2	C
(B)						
CO <sub>2</sub>	1	8	0.448	<b>0.012</b>	0.697	0.963
Temp	1	8	0.099	<b>0.020</b>	0.361	0.250
CO <sub>2</sub> × Temp	1	8	0.053	<b>0.001</b>	0.907	0.756
Time	3	24	0.167	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.001</b>
CO <sub>2</sub> × Time	3	24	0.887	0.609	0.566	0.748
Temp × Time	3	24	0.369	0.302	0.511	0.385
CO <sub>2</sub> × Temp × Time	3	24	<b>0.012</b>	<b>0.001</b>	0.712	0.563

consecutive pair of sampling times cannot be easily categorized by treatments or levels of treatments. For example, between F93 and Sp94, fungal-hyphae biomass in the ACAT decreased (from 790 to 567 g m<sup>-3</sup>, respectively) whereas biomass in the three other treatments increased or tended to increase (from 300 to 402, 309 to 482, and 486 to 542 g m<sup>-3</sup>, respectively for ACET, ECET, and ECAT) (Fig. 3). Between Sp94 and F94, the oscillations in biomass tended to separate according to the temperature treatments with an increase found under the AT treatments (from 555 to 1083 g m<sup>-2</sup> for the two sampling times, respectively) while under the ET treatments the values were similar between the sampling times (441–473 g m<sup>-2</sup>). Finally, between F94 and Sp95, relative changes in biomass do not appear to align according to levels of either of the two climate treatment factors. Consistent with what might be respective treatment seasonal oscillations found in the early period of the exposure, biomass in ACAT decreased, and ECET increased. While hyphal biomass in the ECAT and ACET appeared to be increasing earlier in the exposure, biomass in these treatments decreased between F94 and Sp95.

**3.3.1.2. B1 horizon.** CO<sub>2</sub>, temperature and time significantly affected total fungal-hyphae biomass (Table 3). The CO<sub>2</sub> × temperature interaction and the three-way CO<sub>2</sub> × temperature × time interaction also were significant. While the three factors significantly affected biomass in the B1, the two interactions indicated that the fungal-hyphae biomass responses were complicated. After evaluating pairwise comparisons among treatments, no obvious patterns emerged (Fig. 3).

**3.3.1.3. B2 horizon.** Time was the only factor that significantly affected total fungal-hyphae biomass and none of the interactions

was significant. Overall total fungal-hyphae biomass decreased as the exposure continued. Biomass values in F93 and Sp94 (93 and 103 g m<sup>-3</sup>, respectively) were similar to each other and greater than biomass values estimated in F94 and Sp95 (4.7 and 16.7 g m<sup>-3</sup>, respectively); the latter also being similar to each other.

**3.3.1.4. C horizon.** The pattern of responses of total fungal-hyphae biomass in the C horizon mimicked the responses found in the B2 horizon, i.e., only time significantly affected biomass (Table 3). Total biomass declined as the exposure progressed, with biomass in F93 and Sp94 being similar to each other (approximately 80 g m<sup>-3</sup> at each sampling period), and greater than the total biomass estimated in F94 and Sp95 (3.2 and 11.3 g m<sup>-3</sup>, respectively).

### 3.3.2. Active fungal-hyphae biomass

Active fungal-hyphae biomass was affected by time and varied by horizon (Table 3), and the significant time × horizon interaction indicates that the horizons responded differently as the exposure period progressed. There is suggestive evidence that the three-way interaction of CO<sub>2</sub> × temperature × time affected biomass, but as it occurred only in the chamber analysis we chose to ignore this trend.

Overall, active fungal-hyphae biomass initially was low (0.15 g m<sup>-3</sup> in F93) and reached a plateau by Sp94 (Fig. 3, chamber). Estimated median biomass in Sp94, F94 and Sp95 were approximately 6.0, 4.3 and 4.9 times greater than the amount found in F93, respectively. Pairwise comparisons indicate that estimated biomass values at the three latter times were significantly greater than the amounts estimated for the first



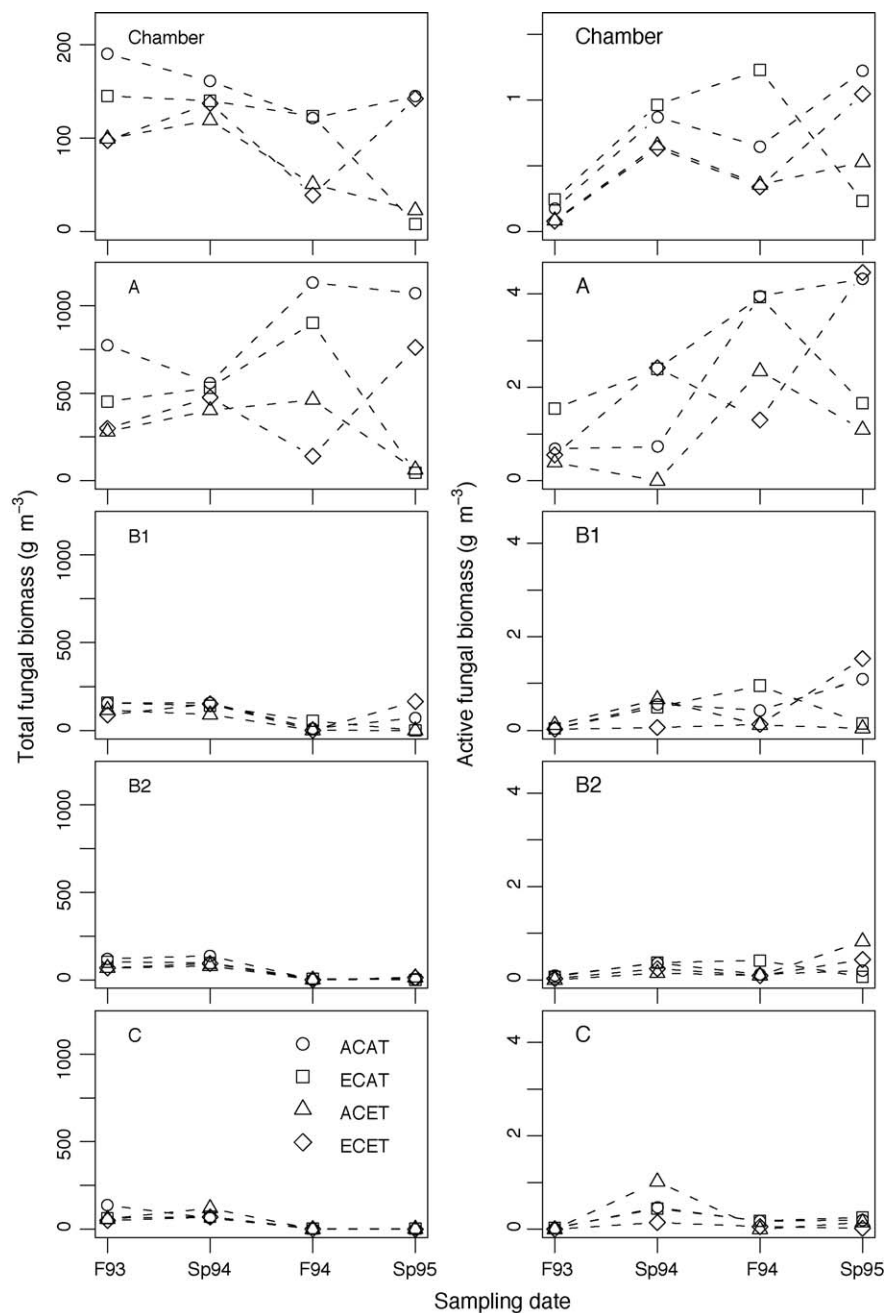


Fig. 3. Estimated median (3 observations/point) total (left) and active (right) fungal biomass. Details and Notes as described in Fig. 2.

sampling, and were not different from each other. The trend of temperature influencing biomass is most clearly seen in the chamber plot of Fig. 3 where biomass appears to be higher in the two AT treatments, especially for the first three sampling times. Overall, the estimated median value for active fungal-hyphae biomass in the AT is 1.6 times than that estimated in the ET treatment (90% CI from 1.05 to 2.45). The temperature trend is less apparent for each of the individual soil horizons where the three-way interaction of CO<sub>2</sub> × temperature × time becomes more evident.

The A horizon contained the greatest amount of active fungal-hyphae biomass during the exposure period (1.29 g m<sup>-3</sup>) while the deeper horizons contained similar and significantly less biomass (0.22, 0.15 and 0.10 g m<sup>-3</sup>, for the B1, B1 and C horizons, respectively).

### 3.4. Nematodes

Nematodes were enumerated to bacteriovorous, fungivorous, plant-parasitic and predaceous functional groups. Counts for the latter two groups were low, and their responses were idiosyncratic with respect to treatments so results for them are not presented. Even though counts for plant-parasitic and predaceous nematodes are included in the total count value, patterns of responses for total nematodes largely mimic responses of the bacteriovores and fungivores indicating the comparatively low presence of the former two feeding types. Also note that F94 results are not reported as these data were not available for the statistical analyses.

Overall, the three functional groups of nematodes responded very similarly to each other during the exposure (Table 4). Counts

**Table 4**

Results of analyses of variance (ANOVA) on log transformed data of number of total, bacteriovorous and fungivorous nematodes. All other notations as described in Table 1.

Factor <sup>a</sup>	Num DF	Den DF	p-Values					
			Nematodes					
			Total		Bacteriovorous		Fungivorous	
			By horizon	Chamber	By horizon	Chamber	By horizon	Chamber
CO <sub>2</sub>	1	8	0.471	0.681	0.693	0.956	0.744	0.578
Temp	1	8	0.336	0.476	0.299	0.575	0.851	0.512
CO <sub>2</sub> × Temp	1	8	0.940	0.511	0.453	0.451	0.863	0.855
Time	2	16	<0.0001	<0.0001	<0.0001	<0.001	<0.0001	<0.0001
CO <sub>2</sub> × Time	2	16	0.613	0.832	0.074	0.806	0.708	0.654
Temp × Time	2	16	0.759	0.844	0.445	0.895	0.964	0.524
CO <sub>2</sub> × Temp × Time	2	16	0.990	0.845	0.872	0.730	0.942	0.944
Hor	3	72	<0.0001	–	<0.0001	–	<0.0001	–
CO <sub>2</sub> × Hor	3	72	0.147	–	0.880	–	0.075	–
Temp × Hor	3	72	0.822	–	0.903	–	0.705	–
CO <sub>2</sub> × Temp × Hor	3	72	0.770	–	0.335	–	0.954	–
Time × Hor	6	72	0.423	–	0.122	–	0.214	–
CO <sub>2</sub> × Time × Hor	6	72	0.407	–	0.636	–	0.302	–
Temp × Time × Hor	6	72	0.217	–	0.709	–	0.142	–
CO <sub>2</sub> × Temp × Time × Hor	6	72	0.116	–	0.280	–	0.093	–

<sup>a</sup> Data for Fall 1994 were not available for statistical analyses.

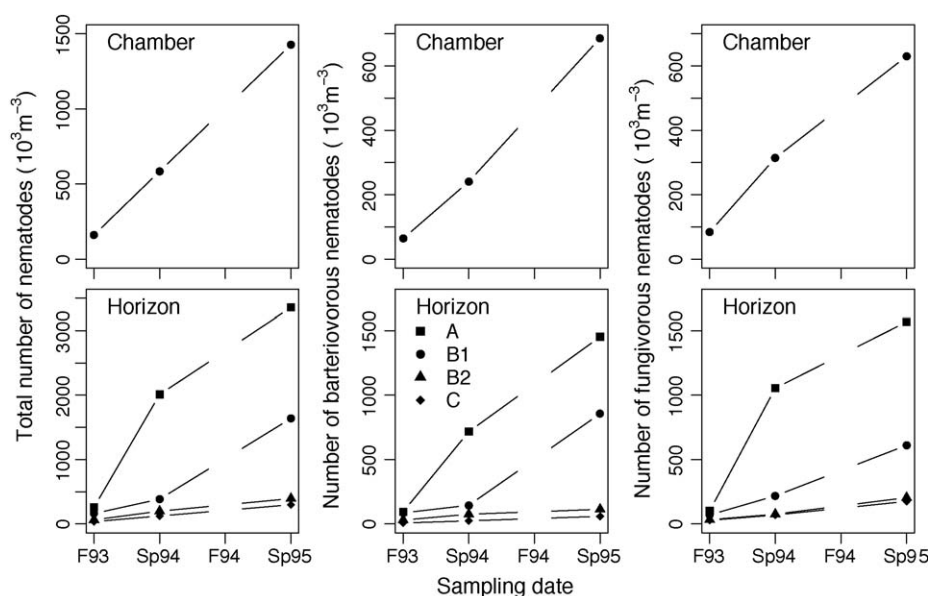
of all groups changed significantly as the exposure continued. Regardless of type, numbers of nematodes were lowest in F93 and relative presence of each type increased as the exposure continued (Fig. 4). More specifically, relative numbers for each group were significantly greater at each subsequent sampling time throughout the exposure. As data for F94 were not available we cannot determine if a peak may have occurred at this time for any of the functional groups. The rate of increase in counts between F93 and Sp95 showed a nonsignificant trend of greater counts for the bacteriovorous than for the fungivorous nematodes (10.7-fold vs. 7.5-fold increases, respectively).

Nematode counts also varied significantly by horizon. Overall, relative estimated median counts by horizon for all three groups followed a similar pattern, although for the bacteriovores for one horizon pairwise comparison, the difference appears to be a nearly significant trend. For total and fungivorous nematodes, estimated median counts were greatest in the A horizon followed by values for the B1; values for the B1 were greater than those calculated for the two lower horizons which were not different from each other.

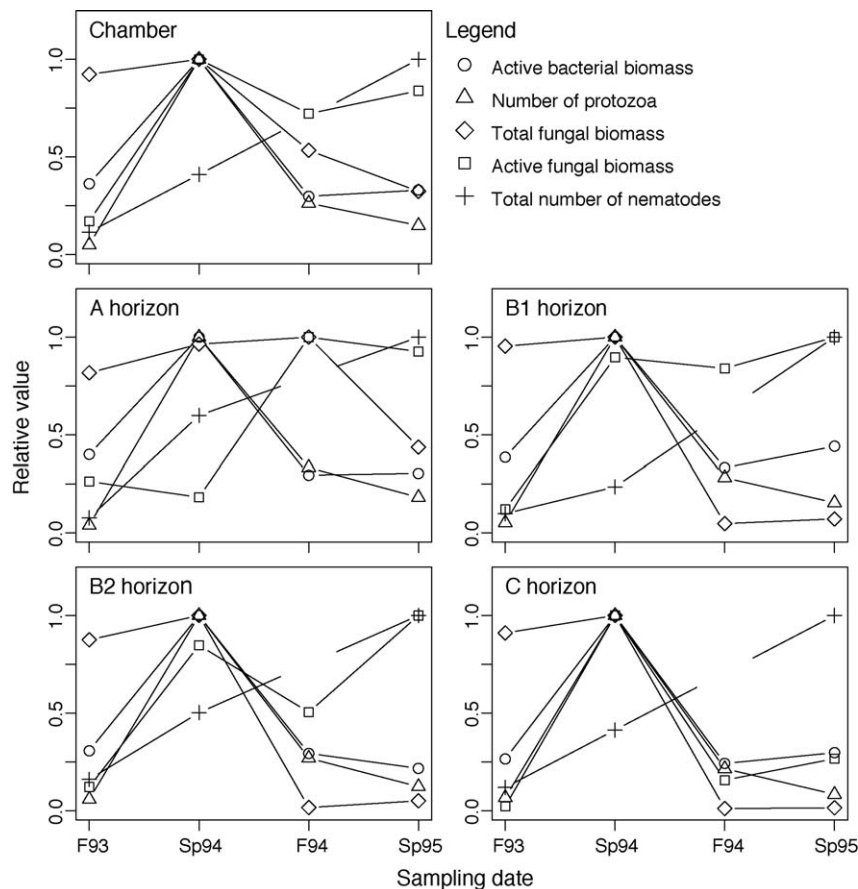
The pattern for the bacteriovores was identical, except that the counts in the A were almost significantly different for counts in the B1. Total nematode counts were  $1212 \times 10^3$ ,  $472 \times 10^3$ ,  $174 \times 10^3$  and  $114 \times 10^3 \text{ m}^{-3}$  for the A, B1, B2 and C horizons, respectively. Values for the fungivorous nematodes were  $563 \times 10^3$ ,  $215 \times 10^3$ ,  $82 \times 10^3$  and  $72 \times 10^3 \text{ m}^{-3}$ , while comparable values for the bacteriovores were  $472 \times 10^3$ ,  $225 \times 10^3$ ,  $65 \times 10^3$  and  $24 \times 10^3 \text{ m}^{-3}$  (all respectively as above).

### 3.5. Changes in relative presence of food web populations

Two patterns emerged describing the temporal dynamics of the populations colonizing the soil (Fig. 5). At the chamber level, presence of total and active bacterial biomass, total and active fungal-hyphae biomass, and amoebae and flagellates peaked in Sp94 followed by a decline in F94, and with the exception of active fungal-hyphae biomass, finished with a relatively less steep decline in Sp95. Nematodes apparently peaked later than did the former populations. We are unable to determine when



**Fig. 4.** Estimated median (12 observations/point) counts of total (left), bacteriovorous (center) and fungivorous (right) nematodes. Details and Notes as described in Fig. 2. Additional Note: Data for Fall 1994 were not available.



**Fig. 5.** Relative changes in food web populations as the climate exposures progressed. Ratios (12 observations/point) for each group are expressed relative to the greatest value observed which was set at the value of 1. The relative changes for total and active bacterial biomass were similar so only the data for active biomass are shown. Similar results were found for number of amoeba and flagellates, and functional groups of nematodes so data for total numbers of protozoa and nematodes are presented. Patterns of total and active fungal biomass varied from each other in all horizons so data are shown separately. *Note:* Nematode data for Fall 1994 were not available.

nematode presence may have peaked during the 20-month exposure owing to lack of data for F94. The two temporal patterns found at the chamber level generally were also found in the four horizons; the notable exceptions are the relative changes in fungal biomass. Unlike total fungal-hyphae biomass in the other three horizons, total biomass in the A horizon did not peak in Sp94, but continued to remain high in F94. Overall, active fungal-hyphae biomass was the most variable. Depending on the horizon, biomass remained relatively low early in the exposure (i.e., A horizon), and later in the exposure biomass either remained high (A horizon) or oscillated seasonally (B1 and B2).

Relative bacterial biomass peaked in the Sp94. Compared with the peaks in Sp94, relative values for active bacterial biomass prior to that date were greater than comparable values for total bacterial biomass with the rankings being reversed after Sp94 (data not shown). Temporal dynamics for relative values for active and total fungal-hyphae biomass were not similar to those found for bacterial biomass. Prior to the peak in Sp94, relative values for active fungal-hyphae biomass were less than comparable values for total fungal biomass. After Sp94 relative values for active fungal biomass were greater than those for total fungal biomass. The patterns described above for bacterial and fungal biomass generally did not vary among horizons.

Overall, relative values for fungivorous nematodes were consistently higher during the exposure compared to values for the bacteriovores (data not shown). Unlike the similarity among horizons found for relative values of bacterial and fungal-hyphae biomass, patterns for nematodes did vary by horizon.

#### 4. Discussion

Using food web data from a reconstructed ecosystem in controlled-environment chambers, we found that there can be short-term responses that are of sufficient strength that they can hamper interpreting the effects of experimental treatments. Soil food web populations responded differentially during the approximately 2 years of this investigation; the variable responses appearing related to whether the biota are components of the bacterial or the fungal energy channels (Gupta and Germilda, 1988), and their trophic position, e.g., the delayed response of nematodes relative to respective prey populations. There was a consistent, rapid and ephemeral spike in bacterial biomass and their shorter lived consumers. The response of fungal biomass was more varied and comparatively more muted (Fig. 5, Chamber). The longer lived predators, i.e., nematodes, were delayed in response as might be expected when resources are passed between trophic levels (Adl, 2003, p. 165). While there are published food web data for Douglas-fir forests along the west coast of North America (e.g., Cromack et al., 1988; Ingham and Thies, 1996) we are not aware of any published data where the soil was handled in similar ways and with a similar schedule to that done in here.

The temporal pattern of bacterial biomass is consistent with bacteria consuming increased amounts of newly exposed C (Davidson and Ackerman, 1993; Wardle, 1995) that became available when soil aggregates were disrupted because the soil was handled. As one might expect, the shorter lived bacterial predators (amoebae and flagellates) mimicked the pattern of bacterial

biomass (Swift et al., 1979). In general, the temporal patterns for these biota at the chamber level were found in the four master horizons indicating a widespread response. It is interesting to us that while the B1, B2 and C horizons were placed in the lysimeters in 1992, and the A horizon was placed in 1993, the general temporal patterns of responses of the biota were similar among horizons. We are not certain why a consistent pattern among horizons occurred, but we have two possible explanations. The first explanation involves the production and movement of dissolved organic carbon (DOC) due to the addition of fresh A horizon and litter layer in 1993 (A horizon ~2.6% C, litter layer ~39.3% C). Collecting, sieving and placing these materials in the lysimeters, followed by repeated watering of the seedlings, probably allowed DOC to be leached into the deeper soil horizons which then became substrate for the soil food web. Release of DOC from forest soils usually is small in comparison with losses of C through respiration (Borken et al., 1999), and can be sufficient for respiratory responses in deep soil horizons (Chow et al., 2006). Our watering regimen of at least twice per week provides further support for this interpretation. Fierer and Schimel (2002) and Merckx et al. (2001) demonstrated that wetting and drying of soil layers, particularly those rich in organic matter, provides a mineralizable source of DOC. DOC becomes substrate for microbial respiration, with greater amounts of DOC being released in the earlier watering events with decreasing amounts found with subsequent watering events (Lundquist et al., 1999). The specific form of this overall pattern appears to be a function of the extent of drying between watering events. If the soil stays moist, adding water releases a steady and low amount of DOC. If the soil becomes very dry, adding water releases a greater pulse of DOC. Temperature also is a factor. Christ and David (1996) reported that DOC release from a forest floor in the state of Maine increased with temperature. In contrast, Chow et al. (2006) found that DOC production was independent of temperature in agricultural soils on the San Joaquin Delta, California. It seems that the DOC response to temperature depends upon the soil and is not easily generalized. If our system behaved more like that reported by Christ and David (1996) then watering events in the summer likely release more DOC than during the winter months. Our soil solution analysis system was not online during the early part of the experiment, so we do not have any direct measures of DOC for this period. Consequently, our inferences concerning DOC are speculative, but are consistent with results reported in the literature. The second explanation involves both the role of DOC released from the litter layer and A horizon, and a temporally overlapping release of C from another source. Prior to placing the A horizon and litter layer in the lysimeters, and planting the seedlings, the B1, B2 and C horizons supported the growth of cover crops. Labile C also may have been made available as the roots of the cover crops continued to decompose during the period under study herein.

We examined both the total and active fungal hyphae in this study. Active fungal hyphae are the subset of fungal hyphae that have active enzymes (as measured by fluorescein diacetate hydrolysis activity). Fungi are capable of economizing the cytosolic contents of much of their hyphal length, concentrating these resources at the growing tip where extra-cellular digestion of substrate primarily occurs. The depleted hyphal lengths then serve as microcapillary conduits integrating environmental conditions and allowing fungal metabolic functions that would not be efficient if they relied only on the resources in the local soil microsite. This is also the basis of the mycorrhizal mutualism between plants and fungi (Adl, 2003, pp. 194–197). Hobbie et al. (2007) indicated that C was allocated to at least two types of fungal hyphae, saprotrophic and mycorrhizal, in an analysis of C consumption by food web organisms at the final harvest of the study. In our previously reported analysis of ectomycorrhizas

(EcM) in this experiment (Rygielwicz et al., 2000) fungi of the genus *Rhizopogon* were consistently dominant in number of EcM root tips for the period Sp94 to Sp97. The AT treatments significantly increased numbers for *Rhizopogon* in the A horizon compared with the ET treatments and this effect was accentuated in the fall samplings giving a consistent seasonal pattern. *Rhizopogon* were also dominant in EcM in a prescreening sampling in F93, and these EcM were common on the seedlings when they were planted. The dominance of *Rhizopogon* EcM was particularly pronounced in F94, with a significant temperature effect for both relative and absolute numbers in the A horizon and for absolute numbers in the B1 horizon. In the present study we found a similar effect for both total and active fungal hyphae with greater biomass in F94 for the AC treatments in the A horizon. The delayed pulse for the A horizon, seen in high values for the AT treatments in F94, may represent the influence of the *Rhizopogon* EcM fungi that had colonized the soil soon after planting. It appears that any saprotrophic pulse was subsumed into the later pulse of EcM affiliated hyphae.

The highly significant effects for treatments, time and the corresponding interactions seen for total fungal-hyphae biomass in the B1 are difficult to decipher. The trends we can see are a decline after the Sp94, similar to the two horizons below, with the exception of a subsequent rebound for the ECET and ACAT treatments Sp95. This would likely produce the interactions we see. The intricate pattern for active fungal-hyphae biomass in the B1 results from the influence of two notably higher biomass values obtained from cores harvested from two chambers Sp94.

Nematode presence generally lagged behind the temporal patterns observed for their prey. This was clearly evident for the bacteriovorous nematodes relative to the spike in bacterial biomass. Even though the temporal patterns for fungal biomass were variable between total and active forms, and among the master horizons, the temporal pattern for fungivorous nematodes was uniform, generally mimicking the patterns observed for the bacteriovores. For both feeding groups, count data for the master horizons reflected the relative amounts of prey found in each horizon. We were unable to ascertain whether the nematodes reached peak numbers in F94 or sometime later as the data for that sampling time were not available, but the relative temporal lag in the presence of nematodes further supports our assertion that the soil food web was responding to the pulse of C released due to reconstructing the soil profile in the lysimeters.

## 5. Conclusion

Our results summarized in Fig. 5 show strong temporal relationships between predator and prey dynamics. Generally, we found a spike in the presence of most food web populations 1 year after completing the installation of the soil in the lysimeters and starting the climate exposures. Notable exceptions are nematodes and fungal-hyphal biomass. The similar temporal spike observed in all the horizons occurred when the majority of fine roots (<2 mm diameter) were found in the A and B1 horizons. The percentage distribution of total fine roots in the cores harvested for food web analyses were 37, 41, 17 and 5 (means for the four climate treatments) for the A, B1, B2 and C horizons, respectively (unpublished data). This suggests that direct root–soil food web interactions, e.g., root exudation, may have played a relatively small role in affecting food web populations. While climate treatments significantly affected populations, their responses to treatments were idiosyncratic within and among the functional groups, except for total bacterial biomass and total fungal-hyphae biomass. Fungal-hyphae biomass appeared affected seasonally by the temperature treatments and this may be due to the complexity of the fungal community, i.e., both mycorrhizal and



saprotrophic fungi were present, and that the seedlings as received from the nursery were colonized with mycorrhizal fungi. Considering the strong temporal patterns found for food web populations coupled with data previously collected on proportioning soil CO<sub>2</sub> efflux into its source components, it appears that the food web initially responded to changes in availability of soil C caused by relocating and reconstructing the soil rather than to the climate treatments. And for some populations in certain horizons the recovery period lasted at least 2 years. We conclude that knowing the length of time needed for such pulses of newly available C to be processed through the food web is critical to understanding the role of ecosystems as net sources or sinks for increased concentrations of atmospheric CO<sub>2</sub>. It is also critical for determining the length of time to maintain studies involving reconstructed ecosystems before results are obtained representative of systems responding to experimental treatments. It is critical to know such information to make informed inferences for any experiments utilizing soil that was disturbed in some way.

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